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ON SOME ANALOGIES BETWEEN THE
PHYSIOLOGICAL EFFECTS OF HIGH
TEMPERATURE, LACK OF OXYGEN,
AND CERTAIN POISONS

A DISSERTATION SUBMITTED TO THE FACULTIES OF THE GRADUATE
SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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WILLIAM D. ZOETHOUT

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ON SOME ANALOGIES BETWEEN THE PHYSIOLOGICAL EFFECTS OF HIGH TEMPERATURE, LACK OF OXYGEN, AND CERTAIN POISONS.

By WILLIAM D. ZOETHOUT.

[From the Hull Physiological Laboratory of the University of Chicago.]

DR. LOEB has expressed the opinion in his lectures that high temperatures and other strong stimulations affect the organism in the way that lack of oxygen does, and it was with the intention of testing the correctness of this supposition that the following series of experiments was made.

The theory that strong stimulation acts like the want of oxygen is based upon Hoppe-Seyler's theory of oxidation.¹ In the cell processes of fermentation take place, by means of which highly reducing substances such as nascent hydrogen are formed. These readily unite with atmospheric oxygen, if it be present, forming oxidized products ($H_2 + O_2 = H_2O + O$) and setting free atoms of oxygen. The free atoms of oxygen are then capable of oxidizing other substances (sugar, lactic acid, etc.) at the low temperature of the animal body. But if oxygen is not present, the nascent hydrogen can reduce the substances in the cell and thereby form new products normally not found in the body. The bodies thus formed may be more or less injurious; in fact, they may act as violent poisons.

It is by means of this theory that Dr. Loeb accounts for the difference which he found in the behavior of the heart of *Fundulus* and *Ctenolabrus* when deprived of oxygen.² The heart of a *Fundulus* embryo beats normally about 120 times a minute at a certain temperature. If deprived of oxygen, the rate is reduced within less than an hour to twenty beats, but the heart continues beating at this rate for over twelve hours. The embryo heart of *Ctenolabrus*, on the other hand, if deprived of oxygen, after a few minutes stops almost suddenly without any considerable previous reduction in the rate. Thus the two hearts differ to a remarkable extent. The stopping of the *Ctenolabrus* heart can hardly be due to lack of energy, for the

¹ HOPPE-SEYLER: *Physiologische Chemie*, 1878, i, pp. 126 *et seq.*

² LOEB, J.: *Arch. f. d. ges. Physiol.*, 1896, lxii, pp. 249-294.

muscles of the body still contract after the heart has ceased to beat. In the case of the *Fundulus* heart, energy for the slow contractions, which continue many hours without oxygen, probably is furnished by fermentation — the more rapid beat, soon reduced by lack of oxygen, is no doubt the result of oxidation; but the stopping of the *Ctenolabrus* heart is probably not to be explained by the want of material for fermentation. The most reasonable explanation of the difference in the behavior of the two hearts toward lack of oxygen was furnished by Loeb from experiments on the eggs of the two fish in their early stage of cleavage. When oxygen is lacking, substances are formed in the egg of *Ctenolabrus* which prevent it from segmenting and which dissolve the cell-walls already formed. In the egg of *Fundulus* this does not take place, but the egg goes on segmenting for many hours.

It seems probable that such substances are formed in the embryo heart as well as in the egg of *Ctenolabrus*. The ultimate source of energy of vital action is chemical, but to appear as heart-beat this chemical energy (produced by fermentation and oxidation) must be transformed into molecular energy. To obtain this, certain conditions of protoplasm must exist, and these conditions are normally present in the living heart-muscle. But in the absence of oxygen substances are formed by fermentation which may and most likely do produce grave changes in the protoplasm, — such as changes in surface tension, in osmotic pressure, in the spreading phenomena, — and these changes would prevent the transformation of the liberated chemical energy into that molecular energy required for specific physiological activity.

It may be added that the injurious bodies may be formed in two ways. First, they may be the direct products of fermentation. In this case they would be oxidized, if oxygen were present, and thus rendered harmless; but if oxygen were absent, they would retain their original form, and might produce destructive results. Secondly, these bodies may arise from fermentation secondarily, in consequence of the fermentation products reducing material normally present in the cell and thus making it harmful. This action will never occur if sufficient oxygen is supplied. It may further be added that if Hoppe-Seyler's theory of oxidation is correct, oxygen not only furnishes energy by oxidation but plays an equally important rôle in the synthetical action by which complex bodies are built up for undergoing fermentation.

From these considerations it is evident that death by lack of oxygen may be due to one of two causes: (1) Too little reserve material for fermentation. Here the necessary energy is almost directly dependent upon oxidation, and if the oxygen is not supplied regularly, the death of the organ or organism results almost instantly. (2) To the formation of substances that render the transformation of chemical into molecular energy impossible (*Ctenolabrus*).

We said there seemed to be reasons for believing that high temperature and strong stimuli in general act as does the lack of oxygen. As by stimulation the processes of fermentation are increased, it is evident that if the stimulation be severe and long continued, the fermentation may be so great that the oxygen supply will be insufficient to oxidize all the reducing substances formed. Hence the same conditions would obtain as in lack of oxygen, and death resulting from high temperature could be referred to one of the above two causes.

It was with the object of testing this theory that, at the request of Dr. Loeb, the following experiments were made. Our investigations comprise (1) The effect of high temperature, (2) of lack of oxygen, and (3) of poisons upon the common infusoria, *Paramœcium aurelia*.

HIGH TEMPERATURE.

The experiments on the effects of high temperature were conducted in the following manner. On a warm stage was placed a glass dish, ten centimetres long, six and a half wide, and three deep, half-filled with water heated to the desired temperature. The *Paramœcia* were placed in vessels of two different kinds, — the first was a closed dish, holding thirteen to fourteen drops, and admitted no air; the second differed from the first chiefly in that air was admitted. These dishes were so constructed that their contents, having a small bulk and a large area, and being separated from the heated water by thin glass, very speedily acquired the temperature of the surrounding medium. In order to allow for the cooling of the water by the vessel, the water was always heated one degree above the desired temperature, and this was found to balance the cooling quite accurately. The desired temperature of the water was maintained by a regulated gas flame.

(a) **Different temperatures.** — The closed dish (not admitting air) was filled with *Paramœcium* culture and placed in water of the desired temperature. Column 1 of Table I gives the length of time in minutes required to kill all the infusoria. As the resistance of *Paramœcia*

varies slightly and as it is impossible always to obtain exactly the same conditions, it is not surprising that the series is not perfectly uniform. From these experiments it follows that the duration of life does not decrease in proportion to the rise in temperature, but decreases more rapidly.

Similar experiments were made in the open dish admitting air. The results are found in Column 2 of Table I. From this table it will be seen that the figures corresponding to 36° and upwards are practically the same in Columns 1 and 2. From 36° downwards, however, they vary widely. To what is the difference due?

We think it is because in the one case no air is present (except that absorbed by the water) while in the other, air is admitted. Although the death of *Paramœcia* at 28° and 30° is not directly due to the lack of oxygen, still this want is an important factor, as is shown by the fact that *Paramœcia* placed in the open dish under water having room temperature (15° – 20° C.) suffered no change in 17 hours, while *Paramœcia* placed in the closed vessel (not admitting air), at the same temperature and for the same length of time, were nearly all dead. From these experiments we may draw the conclusion that during the short time that *Paramœcia* live at a temperature of 36° C., the lack of oxygen makes itself felt very little, compared with the effects produced by the high temperature; below 36° C., however, the lack of oxygen is very apparent.

(b) **Heat and concentration of liquid.** — It has been held by some authors that acclimatization to high temperatures is brought about by loss of water from the organism. Davenport and Castle¹ hold this view, for they say: "We are accordingly led to inquire whether the process of acclimatization to higher temperature may not find its cause in the reduction of the amount of water in the chylema spaces, — the organism becoming in this respect more like the spores in which the quantity of free water is reduced to a minimum." It is true that in some physiological phenomena loss of water has the same effect as a lowering of temperature. Loeb² found that the sense of heliotropism of Copepods and *Polygordius* may be changed from negative to positive by lowering the temperature or by the loss of water caused by increasing the concentration of the sea-water. The positive heliotropism can be changed to negative by increasing

¹ DAVENPORT and CASTLE: *Archiv für die Entwicklungsmechanik der Organismen*, ii, p. 227.

² LOEB: *Arch. f. d. ges. Physiol.*, liv, p. 81.

TABLE I.

Duration of life of Paramœcia at different temperature in open and closed dishes and in different solutions.

Temperature.	DURATION OF LIFE IN MINUTES.				
	1 In Paramœcium culture. Closed dish.	2 In Paramœcium culture. Open dish.	3 In $\frac{1}{2}$ % NaCl solution. Closed dish.	4 In $\frac{1}{4}$ % NaCl solution. Closed dish.	5 In $\frac{1}{8}$ % NaCl solution. Closed dish.
17°C. . . .	1560				
20			185	237	
22			87	165	
24			72	82	144
26			36	45	146
28	115		24	39	73
30	100		24	25	72
31		459			
32		362	11	16	58
33		268			
34		154	9	7; 9	26
35	48	121	9		
36	31	38; 31		11	17
37	24	22	7	8	
38	13	19			10
39		14	4	6	
40	12; 9; 13	12	$2\frac{1}{2}$		6
41				4	
42	8	9			
43		7			
44		$4\frac{1}{2}$			

the water in the cell (lowering concentration) or by raising the temperature. In some unpublished researches on *Fundulus* eggs, prior to the publication of Davenport and Castle, Loeb investigated the

same question which they discuss. He found, however, that by raising the concentration of the sea-water, the eggs were not able to withstand as high a temperature as when in normal sea-water.

It was with this question in mind that we made a series of experiments, employing sodium chloride solutions, having the strength of 0.5, 0.25, and 0.125 per cent. The experiments were performed by carefully placing thirteen drops of the required sodium chloride solution in the closed vessel and adding one drop of Paramœcium culture. The results are found in Columns 3, 4, and 5 of Table I. It is very evident, from these experiments that with the increase of the concentration of the solution in which the Paramœcia live their power of resistance to high temperature is decreased. Even a $\frac{1}{8}$ per cent sodium chloride solution considerably decreased their duration of life when subjected to high temperatures.

(c) **Acids and alkalies.** — We next made a series of experiments with acids and alkalies to ascertain whether the reaction of the fluid in which the infusoria were heated had any effect upon their power of resistance to high temperature. Isotonic solutions of sodium hydrate, hydrochloric acid, and sodium chloride were made. The dish was nearly filled with a certain per cent solution of alkali, acid, or sodium chloride, a drop of Paramœcium culture was added, and then the dish was heated in the water-bath to the required temperature. Table II gives the results obtained in some experiments with sodium hydrate solution, the Paramœcia being heated to 40° C.

TABLE II.
Temperature 40° C. — Open dish (air admitted).

Medium.	Duration of life in minutes.	Average in minutes.	Increase over water in minutes.	% Increase over H ₂ O.
Culture	22; 22; 25; 23; 22	23		
Distilled Water .	{ (21; 25); 12; 17; } { 16; 16; 18; 19; } { 17; 13; 14; 17 }	17		
$\frac{1}{400}$ % NaOH . .	31; 31	31	14	83
$\frac{1}{800}$ % NaOH . .	{ (35; 39); 26; 30; 30; } { 26; 27; 25; 28; } { 23; 24; 27; 27 }	28.2	11.2	66
$\frac{1}{1600}$ % NaOH . .	26; 29	27.5	10.5	62

From this table it is evident that a weak sodium hydrate solution ($\frac{1}{4000}$ – $\frac{1}{16000}$ %) increases the power of resistance of *Paramœcia* to the destructive effects of high temperature. This result was verified by many subsequent experiments. The result of one lot comprising about sixty experiments is represented graphically in Fig. 1. In

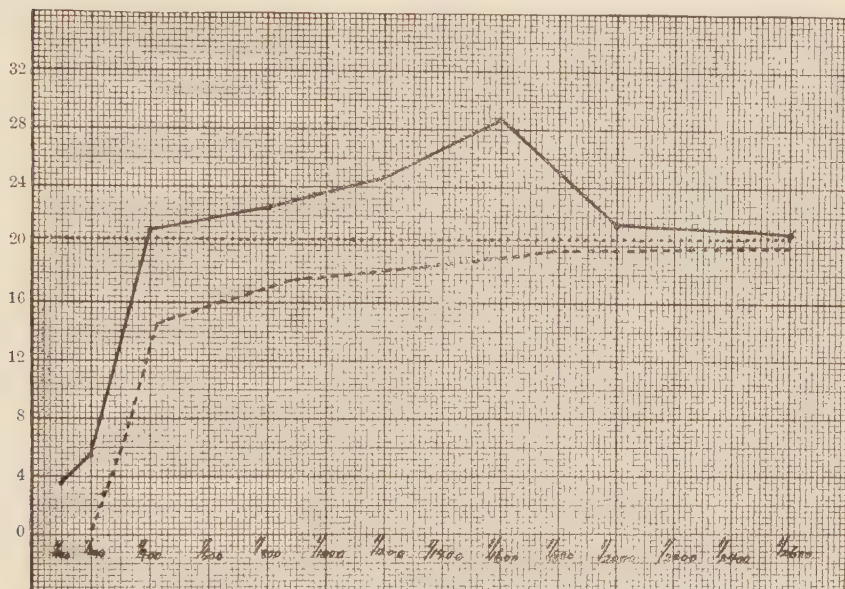


FIGURE 1. Showing duration of life of *Paramœcia* in acid (broken line) and alkali (unbroken line) solutions, and in distilled water (dotted line), at 40° C. The abscissæ give the per cent of concentration of the acid and alkali solutions; the ordinates give time in minutes.

this figure the degree of concentration of the sodium hydrate and hydrochloric acid solutions is represented by the abscissæ, while the ordinates denote the time in minutes which *Paramœcia* live in these solutions at 40° C. The full-lined curve represents the length of life at 40° C. in the various concentrations of sodium hydrate, while the broken curve denotes the length of life in hydrochloric acid solutions at the same temperature. The dotted line at 20.5 minutes, running parallel with the axis of abscissa, shows the length of time which *Paramœcia* live in distilled water at 40° C. As one sees from this figure, the alkali curve between the concentrations of $\frac{1}{4000}$ % and $\frac{1}{16000}$ % is considerably above the straight line which repre-

sents the duration of life in neutral water. In the acid solutions, however, the duration of life (represented by broken curve) is everywhere below this line, showing the acid solution of all concentrations are injurious if they have any effect at all. If the concentration of the alkali solution is greater than $\frac{1}{400}$ % it is decidedly injurious.

The action of a weak alkali solution is still better seen in the following detailed description of two experiments.

Experiment 207.	Experiment 208.
<i>Temp. 40° C. — Open dish.</i>	<i>Temp. 40° C. — Open dish.</i>
Thirteen drops of distilled water and one drop of <i>Paramecium</i> culture.	Thirteen drops of $\frac{1}{1600}$ % NaOH and one drop of <i>Paramecium</i> culture.
After 10 min. — Movements slower and much rotation.	After 10 min. — Movements about normal.
After 15 min. — Many quiet; others very slow.	After 15 min. — None quiet yet; moving slowly.
After 20 min. — All dead.	After 20 min. — Only a couple quiet; the rest moving slowly.
	After 25 min. — A few more at rest; much like at 15 min. in the water medium.
	After 28 min. — All dead.

These experiments show that the conditions after 25 minutes in a $\frac{1}{1600}$ % NaOH solution at 40° C. correspond very nearly to that after 15 minutes in distilled water at the same temperature.

Now this beneficial effect of the alkali solution might be due (1) to its osmotic pressure, or (2) to its chemical qualities. If the effect is due to osmotic pressure, an indifferent solution, such as sodium chloride, of the same osmotic value ought to have the same effect. From the molecular weights of sodium chloride (58.5) and of sodium hydrate (40) we find that a $\frac{1}{400}$ % NaCl and a $\frac{1}{400}$ % NaOH solution have the osmotic pressure of 7.239 and 10.602 mm. respectively, not taking into account dissociation. A $\frac{1}{1600}$ % solution of sodium chloride and sodium hydrate have the osmotic value of 1.8 and 2.6 respectively. As the increase in duration of life caused by alkaline solutions is found between these two limits, we are chiefly concerned with these percentages, and here the differences of osmotic pressure for the same percentages of sodium hydrate and sodium chloride are so small as to be safely ignored.

Table III gives the averages of the results obtained in the sodium chloride solutions at 40° C.

TABLE III.

Temp. 40° C.—Open dish (air admitted).

Percentage of Sodium chloride.	Duration of life in minutes.
0.7	3.5
0.35	8.0
0.1	16.0
0.05	20.5
$2\frac{1}{100}$	21.6
$4\frac{1}{100}$	19.7
$16\frac{1}{100}$	20.6
0.00	20.0

These experiments prove that a neutral salt solution does not have the power of increasing the resistance of *Paramœcia* to heat. The peculiar influence of sodium hydrate solution cannot therefore be due to its osmotic pressure, but must be due to chemical influences.

In the same manner in which we tried the effect of alkali we also tried the effect of acid solution. For convenience of comparison we employed hydrochloric acid solutions isotonic with those of sodium hydrate. The osmotic pressure of a $2\frac{1}{100}$ % hydrochloric acid solution is the same as that of a $2\frac{1}{100}$ % sodium hydrate solution.

A $17\frac{1}{100}$ % hydrochloric acid solution corresponds to a $16\frac{1}{100}$ % sodium hydrate solution. The results of experiments with hydrochloric acid solutions are represented by the broken curve in Fig. 1. This curve shows that if acids have any effect at all, they have the opposite effect to alkalis, namely, shortening the life of *Paramœcia* subjected to high temperature. Never does a hydrochloric acid solution increase the power of resistance against high temperature, but even such a weak solution as $16\frac{1}{100}$ per cent decreases the duration of life at 40° C. by about five per cent.

We have thus seen that the peculiar effect of alkali in lengthening the life of *Paramœcia* exposed to high temperatures (37–40°) is not due to its osmotic pressure. Again we have seen that acids do not possess this peculiar action. From these considerations we may infer that by the action of high temperature a substance is formed in the *Paramœcia*, which eventually brings about the destruction of the organism. This substance is neutralized by alkali but not by acid. This might lead us still further to suppose that the substance formed is an acid.

LACK OF OXYGEN.

From the theoretical considerations mentioned at the beginning of this paper we concluded that what took place more rapidly at a high temperature might take place more slowly in lack of oxygen, and that therefore alkali would have the same effect as

in the case of high temperature. To determine this we made the following series of experiments on lack of oxygen. The air was eliminated by the usual method — replacement by hydrogen. For this purpose the well-known Engelmann gas-chamber was employed. The hydrogen was carefully washed in a potassium permanganate solution, a concentrated solution of potassium hydrate, and distilled water. Two, and in some cases three wash-bottles of the alkali were inserted, so that the current of hydrogen passed three times through a layer of potassium hydrate solution of about nine centimetres thickness. All these precautions were taken in order to remove every trace of acid which might have been introduced with the hydrogen. In the Engelmann chamber was placed a slip of glass to which were attached two rubber rings thirteen millimetres in diameter, and one millimetre high. The cells thus formed were capable of holding seven drops of fluid. In one of the cells were placed six drops of water and one drop of washed *Paramœcia*, while the other cell held six drops of the alkali solution plus one drop of washed *Paramœcia*. The current was then let in, and the behavior of the animals observed and the time of death noted.

As the alkalinity of the water in the aquarium may be considerable and as the alkalinity and the concentration of the water may vary from day to day, we thought it well to employ "washed" *Paramœcia*. It is well known that the *Paramœcia* in the aquarium collect densely around the edge of the dish, two or three millimetres below the surface. It is therefore easy to collect a great number of the infusoria without taking much of the water of the aquarium. They are then placed in tubes of distilled water. Besides the very slight difference of concentration and alkalinity of the cultures used, the varying strength of the hydrogen current has to be taken in consideration; when the current is strong, the time required will of course be much less than when it is weak. From this it becomes evident that individual experiments are not to be compared with each other. For example, it would be incorrect to compare Experiments 248 and 249.

Experiments 248-254 of Table IV give the results of a series which show that in a weak alkali solution the *Paramœcia* withstand the lack of oxygen for a longer time than in distilled water. The average increase in these four experiments is 116 per cent. Hence the effect of a weak alkali solution in the case of lack of oxygen is the same as in the case of high temperature; in both the resist-

ance of Paramœcia is increased. To show that here also the influence of the sodium hydrate solution is not due to osmotic pressure, we compared the duration of life in water and in a sodium chloride solution. Experiments 387-396 of Table IV give the results of this inquiry. The increase in the sodium chloride solution is very slight compared with the increase found in Exp. 248-254, where sodium hydrate solutions were employed.

TABLE IV.

Duration of life of Paramœcia subjected to lack of oxygen.

No. of Exp.	Duration of life in minutes.					Increase in NaCl over Water.		Increase in NaOH over H ₂ O or NaCl.	
	In H ₂ O.	In NaCl.		In NaOH.					
			% Solu- tion.	Time in min.	% Solu- tion.	Time in min.	In min.	In %.	In min.
248	15			$\frac{1}{800}$	40			25	170
249	65			"	107			42	70
252	20			"	42			22	110
254	33			"	71			38	115
387	50	$\frac{1}{400}$	90			40	80		
388	32	$\frac{1}{100}$	42			10	30		
389	62	$\frac{1}{200}$	66			4	$6\frac{1}{2}$		
390	39	$\frac{1}{10}$	42			3	8		
391	16	$\frac{1}{400}$	13			-3	20		
392	32	$\frac{1}{100}$	36			+4	+32		
393	25	$\frac{1}{10}$	45			20	80		
394	13	$\frac{1}{700}$	18			5	40		
395	11	$\frac{1}{700}$	11			0	0		
396	23	$\frac{1}{700}$	22			-1	$4\frac{1}{2}$		
380		$\frac{1}{100}$	15	$\frac{1}{400}$	95			80	540
381		$\frac{1}{300}$	28	$\frac{1}{400}$	46			18	65
382		$\frac{1}{1600}$	65	$\frac{1}{2000}$	65			0	0
383		$\frac{1}{80}$	11	$\frac{1}{100}$	22			11	100
384		$\frac{1}{160}$	11	$\frac{1}{200}$	39			28	255

To show this still more clearly we compared the effect of an alkali and a neutral salt solution side by side, the results of which are given in Exp. 380-384, Table IV. The solution of sodium hydrate and sodium chloride here used were almost isotonic. Neglecting dissociation, the osmotic pressure of

$\frac{1}{300}$ per cent NaCl solution = 9.65 mm.; of $\frac{1}{400}$ per cent NaOH = 10.6 mm.
 $\frac{1}{100}$ " " " = 18.09 mm.; of $\frac{1}{200}$ " " = 21.20 mm.
 $\frac{1}{80}$ " " " = 36.195 mm.; of $\frac{1}{100}$ " " = 42.40 mm.

These last experiments (380-384) show that the average increase of duration of life in a sodium hydrate solution (above $\frac{1}{2000}$ per cent, over that in the sodium chloride solution is 240 per cent. There is therefore no doubt that in lack of oxygen, as in the case of high temperature, the peculiar effect of alkali solutions is not due to osmotic pressure.

It still remains to show the effects of acid. Table V shows the action of hydrochloric acid compared with water.

TABLE V.

No. of Exp.	Duration of life in minutes.			Increase in HCl.	
	In H ₂ O.	In HCl Solution.			
		% Solution.	In min.	In min.	In %.
385	33	$\frac{1}{438}$	4	-29	-87
386	21	$\frac{1}{438}$	3	-18	-86
398	30	$\frac{1}{875}$	21	-9	-30
"	24	$\frac{1}{875}$	15	-9	-37
"	52	$\frac{1}{1752}$	48	-4	-8
"	46	$\frac{1}{1752}$	45	-1	-2
"	27	$\frac{1}{3504}$	28	+1	+4
"	34	$\frac{1}{3504}$	32	-2	-6

From this table it will be observed that hydrochloric acid solutions, as in the case of high temperature, act unfavorably upon *Paramœcia* in case of lack of oxygen.

To show the favorable action of a sodium hydrate solution in detail, we append the notes on Experiment 247.

Experiment 247.

Paramœcium aurelia subject to lack of oxygen.

A number of the Paramœcia are placed in distilled water; the others, in a $\frac{1}{800}$ per cent sodium hydrate solution.

- | | |
|-----------|--|
| 1.15 P.M. | The hydrogen introduced into Engelmann chamber. |
| 1.50 P.M. | In the distilled water, some are entirely quiet; some of them are rotating rapidly, with no progressive movements. A few of the smaller are still swimming. In the $\frac{1}{800}$ % NaOH about 80 per cent are still swimming in a most lively fashion. The rest are rotating. Those in the water appear much swollen, being about twice the size of those in the alkali. |
| 2.05 P.M. | Hardly any life left in the water. About half of those in the alkali are still moving about quite lively; a few absolutely quiet; the remaining are rotating. |
| 2.15 P.M. | No more life left in the water. About half of those in the alkali are still moving quite lively. |
| 2.35 P.M. | About one quarter of those in the alkali swim quite briskly. |
| 2.45 P.M. | In the alkali a few are still moving slowly. |

Experiment interrupted.

Thus we have shown that alkali solutions of from $\frac{1}{200}$ to $\frac{1}{800}$ per cent increase the resistance power of Paramœcia to both high temperature (40° C.) and lack of oxygen. In this respect we have shown the similarity between the physiological effects of both. We have further shown that this action of the alkali is not due to its osmotic pressure; hence it must be due to its chemical composition. Again it has been proven that acids do not increase the power of resistance to these two agents, but that, if at all active, they have a weakening influence. This might lead us one step further in our theory, namely, that the peculiar action of the alkali in these circumstances is due to the fact that it neutralizes a substance, perhaps an acid, formed by strong stimulation and during lack of oxygen.

POISONS.

Through the observations of Claude Bernard it has been proven that if an animal is killed by potassium cyanide the blood in the veins remains arterial, and Geppert proved that potassium cyanide renders the tissues unable to take up oxygen. Such a manner of death is therefore comparable to that brought about directly by lack of oxygen. If this is correct, we ought to be able to demonstrate in the case of poisoning by potassium cyanide, the peculiar action of alkali that we found in the case of lack of oxygen. With this in mind, we made the following series of experiments.

TABLE VI.

One per cent potassium cyanide; effects of alkali.
10 drops of the liquids H₂O, NaOH, HCl, or NaCl +
1 drop of washed *Paramœcia* plus 1 drop of 1.0% KCN.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H ₂ O	In NaCl $\frac{1}{200}$ %	In NaOH $\frac{1}{200}$ %	H ₂ O	NaCl	H ₂ O	NaCl	
256	28	11	30	2	17	7	160	Average increase in min. over { H ₂ O = 13 NaCl = 12.6 In per cent over { H ₂ O = 118 % NaCl = 103 %
257	36	36	61	25	25	70	70	
258	8	7	28	20	21	250	280	
259	12	14	30	18	16	150	115	
260	8	15	26	18	11	225	75	
261	12	12	29	17	17	140	140	
262	9	19	23	14	4	155	22	
263	7	8	13	6	5	90	80	
264	11	11	16	5	5	45	45	
265	11	11	16	5	5	45	45	Av. increase in min. over { H ₂ O = 11.3 NaCl = 15.0 In per cent { H ₂ O = 162 % NaCl = 272 %
		0.1 %	$\frac{1}{160}$ %					
282	5	5	21	16	16	320	320	
283	7	6	21	14	15	200	250	
284	8	5	17	9	12	115	240	
285	8	5	21	13	16	165	320	
286	15	7	23	8	16	55	230	
287	7	..	15	8	..	115	..	

In a small dish, eight or ten drops of distilled water were placed, to which was added one drop of *Paramœcia* and one drop of the chemical agent to be tested. In a second dish, the water was replaced by a solution of sodium chloride, sodium hydrate, or hydrochloric acid, while the rest remained the same. In most of the experiments three trials were made at once, one in water, one in an

TABLE VII.

One per cent potassium cyanide; effects of acid.
Conditions the same as in Table VI.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H ₂ O	In HCl $\frac{1}{4.88}\%$	In NaOH $\frac{1}{2.00}\%$	H ₂ O	HCl	H ₂ O	HCl	
267	12	10	18	6	8	50	80	
268	6	4	11	5	7	85	175	
		$\frac{1}{8.78}\%$						
269	5	3	7	2	4	40	135	
270	9	8	12	3	4	35	50	
		$\frac{1}{17.52}\%$	$\frac{1}{10.0}\%$					Av. increase in min. over $\left\{ \begin{array}{l} \text{H}_2\text{O} = 4, \text{ or } 58\% \\ \text{HCl} = 4.4 \text{ or } 64\% \end{array} \right.$
272	5	5	8	3	3	60	60	
273	9	8	13	4	5	45	63	
274	7	7	12	5	5	70	70	
		$\frac{1}{12.00}\%$						Av. increase in min. over $\left\{ \begin{array}{l} \text{H}_2\text{O} = 2.7 \text{ or } 29\% \\ \text{HCl} = 3.4 \text{ or } 34\% \end{array} \right.$
275	7	6	7	0	1	0	1 $\frac{1}{2}$	
276	8	8	11	3	3	36	36	
277	10	9	15	5	6	50	65	

If 0.7 per cent sodium chloride is used, death occurs almost instantly. If 0.025 per cent sodium hydrate is used, death occurs in about one minute. The one per cent potassium cyanide has a strong alkaline reaction.

alkali solution, and the third in a sodium chloride solution. The three tests were made side by side for ready comparison, and at exactly the same time.

We first made experiments with potassium cyanide, the results of which are embodied in Tables VI and VII.

TABLE VIII.

One tenth per cent sulphate of Atropine ; effects of alkali and acid.

Eight drops of H₂O, NaOH, NaCl, or HCl solutions plus one drop of washed Paracæcia plus two drops of 0.1 per cent Atropine.

No. of Exp.	Duration of life in minutes			Increase in min. in NaOH over		Increase in % in NaOH over		Average increase in NaOH
	In H ₂ O	NaCl	NaOH	H ₂ O	NaCl	H ₂ O	NaCl	
		$\frac{1}{200}\%$	$\frac{1}{100}\%$					
288	3	3	6	3	3	100	100	Over H ₂ O = 5 min. or 124% Over NaCl = 7 min. or 157%
289	3	4	10	7	6	235	150	
290	14	5	17	3	12	21	240	
291	5	5	12	7	7	140	140	
		$\frac{1}{400}\%$	$\frac{1}{200}\%$					Over H ₂ O = 22 min. or 418% Over NaCl = 20 min. or 307%
292	8	6	26	18	20	225	330	
293	4	6	32	28	26	700	430	
294	6	10	26	20	16	330	160	
		$\frac{1}{800}\%$	$\frac{1}{800}\%$					Over H ₂ O = 3½ min. or 76% Over NaCl = 3 min. or 65%
295	9	4	11	2	7	22	175	
296	4	10	13	9	3	225	30	
297	12	12	22	10	10	85	85	
298	27	28	20	-7	-8	-25	-30	Over H ₂ O = 1 min. or 6%
		$\frac{1}{1200}\%$	$\frac{1}{1200}\%$					
299	21	21	22	1	1	5	5	
300	13	13	14	1	1	7	7	
		$\frac{1}{1600}\%$	$\frac{1}{1320}\%$					
			HCl					
301	2	2	3	The 0.1 per cent atropine has no effect upon litmus. A strong solution, however, is decidedly alkaline.				
302	4	4	4					
303	5	3	7					
304	25	25	25					
305	4	4	4					
		$\frac{1}{418}\%$	$\frac{1}{418}\%$					
			HCl					
306	10		4					
307	11		1					

TABLE IX.

Sulphate of strychnine 0.01 per cent; effects of alkali.
 Eight drops of H₂O, NaCl, NaOH, plus one drop of washed *Paramœcia* plus one drop of 0.01 per cent sulphate of strychnine.

No. of Exp.	Duration of life in minutes			Increase in NaOH over H ₂ O		Average increase of NaOH over H ₂ O or NaCl
	In H ₂ O	In NaCl $\frac{1}{100}$ %	In NaOH $\frac{1}{100}$ %	In min.	In %	
308	5	5	0		—∞	
		$\frac{1}{400}$ %	$\frac{1}{200}$ %			
310	11	11	4	-7	-65	
311	5	3	1	-4	80	-73%
312	5	6	1	-4	-80	
313	10	11	3	-7	-70	
		$\frac{1}{1000}$ %	$\frac{1}{2000}$ %			
317	6	4	4	-2	35	
318	6	6	4	-2	-35	-40%
319	4	4	2	-2	-50	
		$\frac{1}{3200}$ %	$\frac{1}{2000}$ %			
322	5	5	4	1	-20	
323	5	5	5	-0	-0	-9%
324	3	5	3	-0	-0	
325	7	9	6	-1	15	

From these tables it is apparent that a weak alkali increases the power of resistance against potassium cyanide while a neutral or acid solution does not possess this power. In fact, while the extreme weak alkali solution of $\frac{1}{3200}$ per cent still increases the time twenty-three per cent, a solution of $\frac{1}{1752}$ per cent hydrochloric acid either

shortens the time or has no effect whatever. That the action of the sodium hydrate is not due to the osmotic pressure is shown in Experiments 256-265, where the difference of pressure between the sodium chloride and sodium hydrate solutions is but seven millimetres.

From observations made by Rossbach it seems likely that alkaloids may also produce death by rendering the tissues unable to take up oxygen. This led us to test the action of acids and alkalies in case of poisoning by atropine, strychnine, etc. The results obtained with atropine poisoning are embodied in Table VIII.

In the experiments with atropine, the beneficial action of sodium hydrate is still better shown than in the case of potassium cyanide. When it is remembered that the alkaloid is already alkaline in its reaction, it is evident that this action of sodium hydrate is not due to the neutralization of the alkaloid. That this peculiar action of alkali is not due to a general stimulating effect or the neutralization of the poison, the following experiments on strychnine (308-329) prove conclusively.

TABLE X.

Sulphate of strychnine 0.01 per cent; effects of acid.
Conditions the same as in Table IX.

No. of Exp.	Duration of life in minutes.			Increase in NaCl.		Average Increase.
	In H ₂ O.	In NaCl 0.1 %.	In HCl $\frac{1}{438}$ %.	In min.	In %.	
326	5	18	0.0	13	260	Of 0.1 % NaCl over H ₂ O = 8.2 min. or 144 %.
			$\frac{1}{1752}$ %			
327	8	16	7	8	100	
328	11	18	11	7	160	
329	9	14	9	5	55	

Sulphate of strychnine 0.01 per cent has no effect upon the litmus, but a stronger solution is decidedly acid in reaction. If 0.7 per cent sodium chloride is used, the *Paramœcia* perish in one or two minutes.

From these experiments it is apparent that a sodium hydrate solution, instead of exercising a beneficial influence, is highly

destructive. This is so radically different from the trend of all the preceding experiments, that I doubted the purity of the solution. But on testing, I found that the Paramœcia in a $\frac{1}{200}$ per cent sodium hydrate solution lived for more than three hours.

Considering that the strychnine is acid in reaction, while the potassium cyanide and atropine solutions are alkaline, we would *a priori* expect that an alkali ought to be more beneficial when added to a strychnine solution than to the others. Instead we found the opposite true. Most likely the chemical action of the strychnine in the cell is different from that of potassium cyanide or atropine. This corresponds with the difference in the physiological effects of these poisons. But even in some alkaloids having an alkaline reaction, the addition of a weak alkali acts unfavorably, as is shown in Experiments 353-358.

TABLE XI. — VERATRINE.

Thirty-five mg. of veratrine boiled in 10 c.c. of water and filtered.

Eight drops of H₂O, HCl, NaCl, or NaOH solutions plus one drop of washed Paramœcia plus two drops of veratrine.

No. of Exp.	Duration of life in minutes.		Decrease in NaOH (in min.)
	In H ₂ O.	In NaOH	
		$\frac{1}{200}$ %.	
		$\frac{1}{400}$ %.	
353	26	2	24
354	40	2	38
355	28	1½	26½
		$\frac{1}{400}$ %.	
356	41	10	31
357	18	15	3
		$\frac{1}{1752}$ % HCl	
358	36	34	

If a $\frac{1}{400}$ % HCl solution is used, the Paramœcia are destroyed almost instantly. The reaction of this veratrine solution is slightly alkaline.

These experiments show that in case of veratrine poisoning, alkali does not increase the duration of life of *Paramœcia*, but shortens it considerably. Experiments on other poisons are in progress.

When we review the poisons thus far experimented upon we find that their chemical reaction, whether acid or alkaline, does not determine whether the addition of an alkali renders the organism more resistant to its actions or not. The effects of strychnine, although it has an acid reaction, are not counteracted by alkalies. Although potassium cyanide and atropine are alkaline, yet their effects are reduced very markedly by sodium hydrate solutions. It may be further remarked that in no case of poisoning has the addition of an acid increased the resistance of *Paramœcia* against the poison. As in the case of heat and lack of oxygen, acid, if active at all, decreases the duration of life of the infusoria.

THEORETICAL REMARKS.

Some authors¹ have attributed death caused by a temperature between 35° and 40° C. to a process of coagulation. Others suppose that coagulation is not the direct cause of death, but that other besides physical causes may be present. Among those who sought to attribute death produced by a temperature of 35° to 40° C. to chemical, and not only to physical causes, may be mentioned Sachs, Engelmann, and Rossbach.

Finding that the life of plants is destroyed by a temperature much below that of coagulation, Sachs² says, "that the coagulation of the protoplasm is not the only point which determines death at high temperature. . . . So long as outside influences do not exceed a certain amount of force, they are not able to subdue the molecular force [in a cell] which binds together the inner organic structure. But if a force, *e. g.* heat, reaches an intensity which exceeds the molecular force, the molecules are torn from their normal position, and the inner structure which serves as the carrier of life is destroyed without any real changes in the outward form." Roth³ extended our knowledge of death at high temperature by showing that alkali solutions are able to revive cilia in heat rigor. Two years later Engelmann

¹ See KÜHNE: Untersuchungen über das Protoplasma und die Contractilität, Leipzig, 1864.

² SACHS, J.: Flora, 1864, xlvii, pp. 5, 24, 33, 65.

³ ROTH, M.: Virchow's Archiv f. pathol. Anatomie, 1866, xxxvii, p. 184.

confirmed this fact. Speaking of this relation between heat and alkalies he says: "It seems to me that the increase of mechanical activity of the cell and the acceleration of the movements produced by heating, are mostly due to the increase in the physiological metabolism (Stoffumsatz). According to our view, in the cells whose movements are increased by heating, the formation of acids is increased. This increased acid formation can, in part, be the cause of the rigor which sets in at 40° C."¹ Rossbach² thinks that we cannot in general consider coagulation as the cause of the cessation of movements at high temperature.

Our conception agrees somewhat with that of Engelmann, as do also, to a certain extent, the facts found.

Our object, as stated in the introduction, was to endeavor to establish the similarity between the effects of high temperature and lack of oxygen. Heat, below the point of coagulation, by increasing fermentation, sets free chemical energy, which can be transformed into molecular energy, appearing in such vital phenomena as ciliary motion, contracting of vacuoles, etc.

But we have above supposed that if the stimulation be very strong and of long duration, the oxidation would be insufficient to oxidize all the reducing products formed by fermentation, and that these products would act upon the contents of the cell in such a way as to render the change of chemical into molecular energy impossible, thereby producing death. *A priori*, it hardly seems likely that *Paramœcia* in an indifferent solution should die in from thirty to sixty minutes because of lack of energy caused by want of oxygen. But if destructive substances be formed, it is easily seen how a sudden death might occur, when we bear in mind the rapid death produced by potassium cyanide and alkaloids. Now from the fact that alkali increases the resistance of *Paramœcia* to both lack of oxygen and high temperatures, we surmise that heat, below the point of coagulation, brings about death in the same manner as lack of oxygen. It may be supposed that the harmful substances, formed by metabolism at high temperature and in lack of oxygen can in some way be rendered inactive, and in this we believe lies the beneficial action of alkali. In some way or other, either by neutralizing their effects or by attacking the substances

¹ ENGELMANN, T. W.: Ueber die Flimmerbewegung, Leipzig, 1868. See also ENGELMANN: Centralblatt für die medicinische Wissenschaften, 1867, p. 657.

² ROSSBACH, J.: Arbeiten aus der zoologischen und zootomischen Institut in Würzburg, 1874, i, p. 9.

themselves, alkali delays death. Seeing they are rendered harmless by alkalies and not by acids, it seems possible that these products formed by fermentation are acids. This also agrees with the experiments of Gaule,¹ who made the important discovery that a weak sodium hydrate solution (1: 20,000) revived the excised frog heart come to rest in a saline solution. He showed also that such an alkaline solution after being repeatedly driven through the heart underwent a loss in alkalinity, due, no doubt, to an acid formed in the heart muscle.

As to the action of potassium cyanide and atropine, it is to be borne in mind that we do not regard these substances as stimuli, as has been well shown by Rossbach in the paper above referred to. This author also pointed out the similarity of action between lack of oxygen (by hydrogen atmosphere) and alkaloids, and he thinks that we may consider the cessation of oxidation of the protoplasm as the ultimate action of alkaloids. Supporting this view, Dr. Budgett² has shown the striking resemblance between the morphological changes produced in case of lack of oxygen and by alkaloid poisoning upon Paramœcia.

If it is true that these poisons kill by diminishing the power of oxidation, while fermentation continues, reducing substances will be formed, and these substances will have the effects already described. Alkali will therefore have the same influence as in lack of oxygen and high temperature. It may be added that there remains the possibility of the sodium hydrate having some chemical reaction upon the potassium cyanide and atropine, but as the percentage of alkali used is so very small, this becomes highly improbable.

SUMMARY.

Alkalies in very small percentage ($\frac{1}{400} - \frac{1}{2000}$ per cent) increase the resistance of Paramœcia to heat (36–40°C.), to the lack of oxygen, and to the destructive action of potassium cyanide and atropine. Acids (hydrochloric), on the other hand, never increase, but when at all active, always decrease the power of resistance to the agents named.

These facts we try to explain in that the lack of oxygen (produced by hydrogen atmosphere or by poisons preventing oxidation) and

¹ GAULE, J.: *Archiv für Physiologie*, 1878, p. 291.

² BUDGETT: *This journal*, 1898, i, p. 98.

high temperature cause the formation of certain substances which render the transformation of chemical into molecular energy impossible, thereby producing death. These substances are in some way antagonized by alkalies, but not by acids.

I take this opportunity to express my thanks to Dr. Loeb for aid kindly given in the execution of this work.

